Status 09/09/05 I-016-96/3
PATENT

Attorney Reference Number 6395-62064-01
Application Number 10/046,955

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Morrison et al.
Application No. 10/046,955
Filed: January 14, 2002
Confirmation No. 5021
For: NUCLEIC ACIDS FOR DETECTING FUSARIUM SPECIES AND OTHER FILAMENTOUS FUNGI
Examiner: Jeanine Anne Goldberg
Art Unit: 1634
Attorney Reference No. 6395-62064-01

CERTIFICATE OF MAILING

I hereby certify that this paper and the documents referred to as being attached or enclosed herewith are being deposited with the United States Postal Service as First Class Mail in an envelope addressed to: MAIL STOP AMENDMENT COMMISSIONER FOR PATENTS, P.O. BOX 1450, ALEXANDRIA, VA 22313-1450 on the date shown below:

Attorney or Agent for Applicant(s)

Date Mailed September 9, 2005

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DECLARATION OF DR. CHRISITINE MORRISON

UNDER 37 C.F.R. §1.132

1. I, Christine Morrison, am an inventor of the above-referenced patent application. I am employed by the Centers for Disease Control and Prevention, the assignee of the above-identified pending patent application. I hold a Ph.D. degree in medical microbiology, and I had my postdoctoral training in the areas of medical mycology. I have worked on the species-specific identification of fungal species the past eight years. I have expertise in the fields of fungal infections, infectious disease, molecular biology, mycology and microbiology.

2. I have read the specification of the above-referenced application, and the Office action, dated March 11, 2005. It is my understanding that claims 1, 7-8, 24, 30-31, 47 and 49 were rejected as allegedly being obvious over Ligon et al. (PCT Publication No. WO 95/29260) in view of GenBank Accession No. U38558 or GenBank Accession No. U28159 (Duggal et al.) further in view of U.S. Patent No. 5,595,874. However, none of these references provide the necessary information to design probes that allow the species specific detection of *Fusarium*, and

thus do not render the claims obvious. The exact sequence of the ITS2 region of the *Fusarium* species of interest must be known to design species specific probes based on this sequence. In addition, only a subset of probes that are designed based on a *Fusarium* ITS2 sequence will provide a strong reactivity with a single *Fusarium* species of interest without cross-reacting with other *Fusarium* species.

3. Six probes (FMON1, FMON2, FMON3, FMON34, FMON4, and FMON41) were generated based on the *F. moniliforme* ITS2 nucleic acid sequence. One of these probes, FMON34 (SEQ ID NO: 49 in the above-referenced patent application) differed from FMON3 by a single nucleotide. Specifically, FMON34 (SEQ ID NO: 49) had an adenine at position eight, while FMON3 had a guanine at position eight.

Each of the *F. moniliforme* probes was tested for its ability to bind to *F. moniliforme*, *F. oxysporum* and *F. solani* DNA. The ability of a probe to bind *F. moniliforme* DNA was graded on a scale of 0-6, with 6 being the highest score and zero being the absence of a positive signal. An absence of significant binding (scored as a "0") to *F. solani* or *F. oxysporum* DNA indicated that the probe was specific. Strong binding (scored as "4," "5" or "6") to *F. moniliforme* DNA indicated that the probe was sensitive. The results obtained with these probes are summarized in the table provided below.

Probe	Sequence	Binding to *F. moniliforme*	Binding to *F. oxysporum*	Binding to *F. solani*
FMON34	TCTAGTGaGGTCTCGCT (SEQ ID NO: 49)	4	0	0
FMON3*	TCTAGTGgGGTCTCGCT	5	0	2
FMON1	GCAAGCCCTTGCGGCAAG	1	0	0
FMON2	CAAGCCCTTGCGGCAAGC (OFS)	2	0	0
FMON4	AACCCTCGCAACTGGTAC	4	3	1
FMON41	ACCCTCGCAACTGGTACG (OFS)	5	0	0

*sequence difference FMON3/FMON34 shown in lower case, "OFS" indicates offset sequence by one nucleotide

FMON34 (SEQ ID NO: 49 of the above-referenced patent application) showed good positivity (hybridization to *F. moniliforme DNA*) and no cross-reactivity (hybridization with *F. solani* DNA or *F. oxysporum* DNA). FMON3, which differed from FMON34 by only a single

nucleotide, cross-reacted with *F. solani* DNA. Thus, changing the sequence of FMON34 by as little as one nucleotide abolished the specificity of the FMON34.

Other probes were generated from the ITS2 region of *F. moniliforme*. FMON1 and FMON2 reacted only weakly with *F. moniliforme* DNA, and thus were not as sensitive as FMON34 (SEQ ID NO: 49). FMON4 cross-reacted with *F. oxysporum* and *F. solani* DNA, and was not as specific as FMON34 (SEQ ID NO: 49). One other probe, FMON41, showed good positivity and no cross-reactivity. These results demonstrated that FMON34 (SEQ ID NO: 49) and FMON41 provided an unexpectedly superior ability to hybridize with *F. moniliforme* DNA and not to cross-react with *F. oxysporum* or *F. solani* DNA.

4. Seven probes, named FSOL21, FSOL31, FOSL41, FSOL5, FSOL6, FSOL7 and FSOL8, were generated based on the *F. solani* ITS2 nucleic acid sequence. Only three of these probes, FSOL5 (SEQ ID NO: 51 in the above-referenced patent application), FSOL6, and FSOL31 showed strong reactivity to *F. solani* DNA and no cross-reactivity with *F. moniliforme* DNA and *F. oxysporum* DNA.

Specifically, each of the *F. solani* probes was tested for its ability to bind to *F. moniliforme*, *F. oxysporum* and *F. solani* DNA. The ability of a probe to bind *F. solani* DNA was graded on a scale of 0-6, with 6 being the highest score and zero being the absence of a positive signal. An absence of significant binding (scored as a "0") to *F. moniliforme* or *F. oxysporum* DNA indicated that the probe was specific. Strong binding (scored as "4," "5" or "6") to *F. solani* DNA indicated that the probe was sensitive. The results obtained with these probes are summarized in the table provided below.

Probe	Sequence	Binding to *F. moniliforme*	Binding to *F. oxysporum*	Binding to *F. solani*
FSOL21	GCGGGCACAACGCCGTCC	0	0	0
FSOL31	GCACAACGCCGTCCCCCA	0	0	4
FSOL41	CCTGCGGGCACAACGCCG	0	0	1
FSOL5*	CTAACACCTCGCAACTGGAGA (SEQ ID NO: 51)	0	0	5
FSOL6*	ACACCTCGCAACTGGAGA	0	0	5
FSOL7	CTGCGGGCACAACGCCGT	0	0	0
FSOL8	TACAGTGGCGGTCCCGCC	1	0	4

FSOL5 (SEQ ID NO: 51) and FSOL6 showed the strongest reactivity to *F. solani* and the least cross-reactivity to *F. moniliforme* and *F. oxysporum* DNA. FSOL31 also showed good reactivity to *F. solani* DNA with no cross-reactivity with *F. moniliforme* and *F. oxysporum* DNA.

In the *F. solani* ITS2 region, FSOL8 is located in between the sequences represented by FSOL5 or FSOL6 and FSOL7. FSOL8 showed less reactivity to *F. solani* DNA than either FSOL5 or FSOL6 but showed more reactivity than FSOL7. In addition, FSOL8 showed weak cross-reactivity to *F. moniliforme* DNA whereas FSOL5, FSOL6, and FSOL 7 did not show any cross-reactivity.

The results demonstrated that FSOL5 (SEQ ID NO:51), FSOL6, and FSOL31 showed an unexpectedly superior ability to hybridize to *F. solani* DNA and did not cross-react with *F. moniliforme* DNA or *F. oxysporum* DNA.

5. Four probes, each 18 nucleotides in length, were generated based on the *F. oxysporum* ITS2 nucleic acid sequence (FOXY1, FOXY2, FOXY3 and FOXY5). The FOXY1(SEQ ID NO: 50 of the above-identified patent application) is a sequence that is offset in the nucleic acid sequence of the *F. oxysporum* ITS2 region by a single nucleotide from the nucleic acid sequence of FOXY2. Thus, FOXY1 differed from FOXY2 by only two nucleotides. The results obtained with these probes are summarized in the table provided below.

Probe	Sequence	Binding to *F. moniliforme*	Binding to *F. oxysporum*	Binding to *F. solani*
FOXY2	GTTAATTCGCGTTCCTCA	1	4	0
FOXY1*	CGTTAATTCGCGTTCCTC (OFS) (SEQ ID NO: 50)	0	4	0
FOXY3	TTGATTGGCGGTCACGTC	3	5	0
FOXY5	GATTGGCGGTCACGTCGA (OFS)	3	4	0

FOXY1 showed strong reactivity to *F. oxysporum* DNA without any cross reactivity to *F. solani* DNA or *F. moniliforme* DNA. FOXY2, which differed from FOXY1 by two nucleotides, had strong reactivity to *F. oxysporum*, but also weakly cross-reacted with *F. moniliforme* DNA. Both FOXY3 and FOXY5 cross-reacted with *F. moniliforme* DNA. Thus, FOXY1 (SEQ ID NO: 50) showed an unexpectedly superior ability to bind to *F. oxysporum* DNA without cross-reacting with *F. moniliforme* DNA.

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3. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of the Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Christine Morrison
Christine Morrison

09-09-05
Date